Neurology
Autologous mesenchymal stem cells: clinical applications in amyotrophic lateral sclerosis

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Objectives: Our study was aimed to evaluate the feasibility and safety of intraspinal cord implantation of autologous mesenchymal stem cells (MSCs) in a few well-monitored amyotrophic lateral sclerosis (ALS) patients.

Methods: Seven patients affected by definite ALS were enrolled in the study and two patients were treated for compassionate use and monitored for at least 3 years. Bone marrow was collected from the posterior iliac crest according to the standard procedure and MSCs were expanded ex vivo according to Pittenger’s protocol. The cells were suspended in 2 ml autologous cerebrospinal fluid and transplanted into the spinal cord by a micrometric pump injector.

Results: The in vitro expanded MSCs did not show any bacterial or fungal contamination, hemopoietic cell contamination, chromosomal alterations and early cellular senescence. No patient manifested major adverse events such as respiratory failure or death. Minor adverse events were intercostal pain irradiation and leg sensory dysesthesia, both reversible after a mean period of 6 weeks. No modification of the spinal cord volume or other signs of abnormal cell proliferation were observed. A significant slowing down of the linear decline of the forced vital capacity was evident in four patients 36 months after MSCs transplantation.

Conclusions: Our results demonstrate that direct injection of autologous expanded MSCs into the spinal cord of ALS patients is safe, with no significant acute or late toxicity, and well tolerated. The clinical results seem to be encouraging. [Neurol Res 2006; 28: 523–526]

Keywords: Amyotrophic lateral sclerosis; in vitro expansion; mesenchymal stem cells; safety; transplantation

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rapid and progressive neurodegenerative disease that targets motor neurons in spinal cord, cortex and brain stem. The selective degeneration of motor neurons manifests as a linear decline in muscular function eventually resulting in paralysis, speech deficits and dysphagia. Within 2 to 5 years of clinical onset death, owing to respiratory failure, it occurs. There are no therapies available today.

Stem cells therapy holds potential for treating ALS by different mechanisms. Great interest is focused on inflammatory processes and microglia activation in the pathogenesis of ALS. Cells that surround motor neurons and are not nerve cells can play a major role in advancing or limiting the disease¹. Recent research in superoxide dismutase (SOD1) mice has shown that healthy astrocyte and microglia can maintain the health of neighboring diseases motoneurons and greatly extend survival²,³. NeurorDISTENSION itself seems to promote proliferation, migration and transdifferentiation of autologous stem cells⁴. Production of neurotrophic and growth factors and stimulation of the regenerative processes by stem cells have been demonstrated in neurodegenerative diseases⁵. Moreover, stem cells might become eventually carriers of pharmacological treatments. Rats with the mutation responsible for some forms of inherited ALS that have received stem cells engineered to express a supportive factor called glial derived neurotrophic factor (GDNF) did produce encouraging evidence that stem cell implants can live, make connections and secrete GDNF as instructed⁶.

Mesenchymal stem cells (MSCs) from bone marrow (BM) are pluripotent cells that contribute to regeneration of several tissues including the nervous system⁷ through transdifferentiation and cell fusion with the damaged neurons⁸. MSCs have displayed unorthodox plasticity in
their ability to trans-differentiate into non-mesenchymal lineages including astrocytes and myelinating cells of the peripheral nervous system and spinal cord. In vitro, MSCs differentiate into cells expressing neuronal markers when exposed to mitogens such as brain-derived neurotrophic factor and nerve growth factor neurotrophins and retinoic acid, demethylated agents, physiologic neural inducers, antioxidants and compounds which increase intracellular cyclic adenosine monophosphate (AMP). MSCs have been transplanted in different animal models of central nervous systems diseases with evidences of their capability to survive, proliferate and migrate into the damaged tissue with positive functional effects. Recently, it has been demonstrated that BM stromal cells reduce cell death and apoptosis and increase the DNA proliferation rate in astrocytes post-ischemia. Moreover, they can be used as vectors of cytokines and trophic factors preventing cell death, tissue inflammation and damage.

All these evidences highlight the potential use for therapeutic strategies of MSCs in ALS.

This study was aimed to evaluate the feasibility and safety of intraspinal cord implantation of autologous MSCs in a few well-monitored ALS patients.

PATIENTS AND METHODS

The study was approved by the Ethical Committee of the Piedmont Region. Seven patients (four females and three males) were consecutively enrolled from October 2001 to May 2002. Two patients (one male and one female) were treated for compassionate use after approval by the local ethic committee. All patients gave their informed consent. Patients were included if they had definite ALS with spinal onset without signs of respiratory failure. Table 1 reports the main clinical features of patients at entry. Standard therapies were used throughout the study. The patients, in order to estimate disease progression rate before transplantation, had a 6 month period of natural history observation. They were monitored every 3 months by clinical evaluation which included ALS-functional rating scale (FRS), Norris score, bulbar score and Medical Research Council (MRC) strength scale. Respiratory assessment included pulmonary function tests and nocturnal cardio-respiratory monitoring. Neurophysiologic monitoring included electromyography (EMG) and somatosensory evoked potentials. The neuroradiological assessment consisted of magnetic resonance imaging (MRI) of brain and spinal cord before and after gadolinium diethylenetriaminepentaacetic acid (DTPA) infusion. A clinical psychologist assessed quality of life by clinical interview and psychologic tests. After MSC implantation, the patients were monitored for at least 36 months by the same assessment.

Experimental procedures

BM was collected in epidural anesthesia according to the standard procedure. BM cells were layered on a Percoll gradient (density: 1.073 g/ml) and centrifuged at 1100 g for 30 minutes as previously reported. Mononuclear cells at the interface were recovered, washed twice with phosphate buffer saline (PBS) (Cambrex, Walkersville, MD, USA) at 200 g for 10 minutes, seeded at a density of 800,000/cm² in MSC medium (Cambrex) in 150 cm² T-flasks and maintained at 37°C with an atmosphere of 5% CO₂. After 3 days, non-adherent cells were removed and the adherent cells were re-fed every 3–4 days. In order to expand the isolated cells, the adherent monolayer was detached with trypsin/ethylene diamine tetraacetic acid (EDTA) (Cambrex) for 5 minutes at 37°C, after 15 days for the first passage and every 7 days for the successive passages. The cells were seeded at a density of 8000/cm² and expanded for the passages at the latest. At each passage and before implantation MSCs were analysed for viability, sterility, mycoplasma detection and cytogenetic and telomeric analysis according to the guidelines of the Italian Institute of Health and showed by Mareschi et al. Moreover, the surface antigens, CD45, CD14 (Becton Dickinson, San Jose, CA, USA), CD90, CD106, CD29, CD44, CD105 and CD166 (Caltag Laboratories, Burlingame, CA, USA) were analysed on an Epics XL cytometer (Beckman Coulter, CA, USA) with the XL2 software program.

Before implantation the cells were maintained for at least 3 hours in basal MSC medium (Cambrex) without serum, detached and washed there times with PBS (1X) containing 1% human albumin and one time with autologous cerebrospinal fluid. The cells were suspended in 1 ml autologous cerebrospinal fluid and directly transplanted into the surgically-exposed spinal cord at different thoracic levels. A laminectomy was performed and the dura was opened along the median line under microscopic vision. After a median mielotomy, the cells were injected in the most central part of the spinal cord by means of the Hamilton syringe previously mounted in an injection system with a micrometric pump injector supported by a table-fixed arm. At the end of the procedure, the dura was closed in a tight-water faction. The procedure was performed in general anesthesia using short-acting drugs.

The choice to transplant MSCs directly into the spinal cord was made given the impediment of stem cells to cross the blood–brain barrier which is intact in ALS.

Table 1: Clinical characteristics of patients at entry

<table>
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<tr>
<th>Patient</th>
<th>Age</th>
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<th>Bulbar score</th>
<th>ALS-FRS score</th>
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*Compassionate use; FVC=forced vital capacity; F=female; M=male.
RESULTS

BM collection
No side effects (including pain at the posterior iliac crest lasting for 48 hours and infection episodes) were recorded after BM collection.

The median BM collection (without anticoagulant agents) was 607 ml (range: 238–732 ml) and leucocyte collection was 104.4 × 10⁸ (range: 33.3–144.5 × 10⁸). After Percoll separation, there was a median of 11.32 × 10⁸ (range: 4.31–26.79 × 10⁸) counted and seeded cells.

Isolation and implantation of MSCs
The cells used for the implantation were at the second or third passage after a median of 32 culture days (range: 27–34 days). Each harvest revealed a homogenous population of cells, positive for CD29, CD44, CD105, CD166 and CD90 antigens (> 90% of cells) by flow cytometry and negative for hemopoietic antigens CD45 or CD14. All bacteriologic tests performed on the cells at each passage were negative. Detached MSCs showed greater than 95% cell viability before implantation. Cytogenetic analysis did not show any karyotype alterations. No significant shortening related to a cellular senescence was evidenced by the telomere length analysis.

A median of 32 × 10⁶ cells (range: 7.0–152 × 10⁶) was implanted. Table 2 shows the number of MSCs obtained at each passage in relation to the patient’s age.

There were no anesthetic complications. No patients manifested severe adverse events defined as respiratory failure, death and neurological symptoms which persisted for more than 6 weeks after MSC implantation. Minor adverse events were intercostal pain irradiation (four patients) and leg sensory dysesthesia (six patients). No patients manifested bladder and/or bowel dysfunction or leg motor deficit. Serial MRI showed no evidence of structural changes of the spinal cord or signs of abnormal cell proliferation when compared with the baseline also in the long term (3 years after surgery).

Two patients died from the progression of the disease 9 months and 2 years respectively after MSCs transplantation, one patient underwent tracheostomy for respiratory complications owing to ab ingestis pneumonia. A significant slowing down of the linear decline of the FVC (Figure 1) and the ALS-FRS (Figure 2) was observed after transplantation of MSCs in five patients.

DISCUSSION
MSCs were easily isolated from BM in ALS patients. The in vitro cell population was homogeneous with a fibroblastic like morphology and no hemopoietic cell contamination. No cytogenetic alteration or early cellular senescence was observed in the expanded in vitro MSCs used for the implantation. The results of the long-term follow-up appear to confirm that the procedures of ex vivo expansion of autologous MSCs and

<table>
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*Compassionate use.
transplantation in the spinal cord of humans are safe and well tolerated by ALS patients as previously reported.\textsuperscript{19,20}

The long-term follow-up of the patients shows also a significant slowing down of the decline of the FVC and of the ALS-FRS after transplantation in >50% of them. Given the progressive course of the disease, these changes are therefore likely to be due to the positive effect of MSC transplantation. However, given the interindividual variability of the clinical course of the disease and the small number of the treated patients, we cannot speculate about the effects of the MSC intraspinal cord implantation on the natural history of the disease and survival.

Further well-designed clinical studies are warranted to determine its activity in this fatal, incurable disease.

ACKNOWLEDGEMENTS

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In Humans

Treating Parkinson's with Adult Stems Cell and Other Alternatives

Using adult neural stem cells, Dr. Michel Levesque, at the Cedars-Sinai Medical Center in Los Angeles, reports a total reversal of symptoms in the first Parkinson's patient treated. The patient, a 57-year old former fighter pilot, is still without symptoms three years after the adult neural stem cells were removed from his brain, coaxed into becoming dopamine-producing cells, and then reimplanted. Because the stem cells came from the patient, there was no need for immunosuppression to overcome rejection. "I think transplantation of the patient's own neural stem cells and differentiated dopaminergic neurons is more biologically and physiologically compatible - more efficacious and more elegant," said Levesque. In addition to its use for Parkinson's, the technique is under study for juvenile diabetes, stroke, brain tumors, spinal cord injury, and other conditions.¹

Retinal Cell Implants Improve Parkinson's

A team at Emory University School of Medicine has shown that implanting retinal cells into the brains of people with advanced Parkinson's disease can improve motor function by almost half, according to a follow-up study of six patients. The team noted: "We've been following these six participants for over a year, and we've found they've improved, on average, nearly 50 per cent in motor function." The retinal cells used were taken from deceased donors and grown in the lab. The team is not using immunosuppressants.²

In Animals

Stimulating Adult Brain Stem Cells Decreases Parkinson's Symptoms

Injection of growth protein into brains of Parkinson's rats caused their neural stem cells to grow, migrate to the site of damage, and begin to replace missing nerve cells. Eighty percent (80%) of the rats received a benefit from the treatment, with no tumor formation.³

Progenitor Cells Reverse Severe Parkinson's Symptoms in Rats

Researchers at Chicago's Rush University report coaxing progenitor cells from the brains of rats into becoming dopamine neurons to treat Parkinson's disease. Led by Paul Carvey, the team discovered an important "shortcut" to creating a more efficient, more reliable, and safer source of stem cells with the ability to turn into specific neurons or brain cells. This study is the first to identify the signal that instructs stem/progenitor cells to become dopamine neurons. The researchers watched the cells develop, and selected and grew cells that were close to becoming neurons. They then grafted the cells into brains of Parkinson's rats, effectively curing the animals' severe Parkinson symptoms. The ability to select and grow large numbers of adult stem cells that would become neurons also has the potential to revolutionize the treatment of Alzheimer's disease, multiple sclerosis and numerous other diseases and disorders of the brain and nervous system.⁴
Note: In contrast to these animal studies using adult stem cells, a widely publicized study showed just over 50% of Parkinson's rats injected with mouse embryonic stem cells receiving a modest benefit, but one-fifth (20%) of the rats died of brain tumors caused by the embryonic stem cells.  

Gene Therapies Treat Parkinson's in Rats, Monkeys

The injection of two corrective genes into a specific brain region generated significant restoration of normal limb movement in rats with Parkinson's disease. Limb impairments were completely reversed in rats that had near-total Parkinsonian lesions on only one side of the brain, meaning that some of their dopamine-producing cells remained intact. But even in the rats with complete destruction of dopamine-producing cells, the delivery of gene therapy resulted in a limited amount of restored motor function. "We anticipate gene therapy will offer a way to help patients with Parkinson's disease live many years longer free of disabling symptoms," the researchers noted.

A Japanese research team has demonstrated delayed delivery of gene therapy can provide significant recovery from Parkinson's symptoms. Four weeks after inducing Parkinson's damage in their brains, rats were given an injection of a gene vector which produced a growth protein call "glial cell line-derived neurotrophic factor" (GDNF). The animals showed remarkably higher levels of dopamine secretion and significant behavioral recovery, even up to 20 weeks following the injection.

Treatment with three gene therapy vectors has shown behavioral recovery in Parkinson's monkeys. The treatment resulted in remarkable improvement in manual dexterity and restoration of motor functions, with the behavioral recovery persisting for over 10 months in one case. The scientists say that this triple gene therapy method may offer a potential therapeutic strategy for Parkinson's disease.
Stem Cell Therapy Reverses Multiple Sclerosis Damage

By Julie Stachowiak, Ph.D., About.com Guide
January 30, 2009

In a small (very small) trial, stem cell therapy was found not only to stop the progression of multiple sclerosis, but also to reverse damage. This is the first time research has shown that multiple sclerosis can be reversed, not merely slowed. I am cautiously really, really excited about this.

Here are the basics: In the first step of the therapy, physicians take some of the bone marrow stem cells from your body and keep them alive. Then they use chemotherapy to, essentially, wipe out your immune system. After that, they put the bone marrow stem cells back into your body. The new immune system cells produced (by the stem cells) are "naive" and do not see the myelin as an invader, or something to be attacked. Therefore, they no longer attack the myelin and the body (apparently) can then get to work healing any early-stage multiple sclerosis damage.

This study was done in 23 patients, all with early stage relapsing-remitting (RRMS) multiple sclerosis (they have tried this before in people with later stage disease and it was not successful). After one procedure, these folks were followed for three years. 17 of them improved by at least one point on the disability scale and none of them got any worse (in terms of disability). There is a larger trial now underway to learn more about the risks and benefits of this treatment (especially the risks and at what stage of multiple sclerosis is the treatment most beneficial).

For clarification, this uses a procedure called autologous non-myeloablative haemopoietic stem cell transplantation. What that means is that it uses a person's own (adult) stem cells and that the chemotherapy doesn't fully destroy the bone marrow.

As the first approach that is really a treatment, meaning it is healing, and not just slowing progression, this is very exciting. Just to reiterate an earlier point, it is unclear how many people with multiple sclerosis will eventually benefit from this, as it seems to be best in early stage, relapsing-remitting multiple sclerosis. I don't know exactly what "early stage RRMS" means in this context. Does this include people who have lived with RRMS for a long time without too much disability or people who have RRMS that were diagnosed recently, but have had several harsh relapses since diagnosis? Let's all keep an eye on this one. It seems like the past week or two have just been filled with good news, potentially very good news for some people with multiple sclerosis (MS). First we saw the announcement of an oral multiple sclerosis treatment that may be available pretty soon, and then we saw the first actual reversal of multiple sclerosis symptoms.

I am happy to say that the good news continues to come in. At Johns Hopkins University, there is an ongoing project that is (in my opinion) genius in the approach. Pharmacologists (led by Dr. Jun O. Liu) are digging through a huge database of over 3,000 drugs looking for new uses of old drugs. This investigation of existing, known (and known to be safe) drugs for additional purposes represents some of the smartest research out there.

Here's the really good part (for us MSers) - they found one that may help with multiple sclerosis. The drug clofazimine was developed in the 1890s as a treatment for tuberculosis. Clofazimine happens to also interfere with a molecular pathway that controls the immune response. Basically, clofazimine prevents signaling from the exterior of an immune cell into the interior (where it can "rev up" a
response). They result? An inhibited immune system that is less likely to attack the myelin and cause the symptoms of multiple sclerosis (in theory).

I love this line of research, not only because it holds promise for multiple sclerosis, but because it is, in essence, "repurposing" old drugs with known safety measures for new problems. This makes so much sense.

The full study is available online from the Public Library of Science at http://www.plosone.org/article/info:doi/10.1371/journal.pone.0004009